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August 14, 2003

VIA EXPRESS MAIL, WITH RETURN POSTCARD ENCLOSED

PCT International Application Processing Div.
USPTO International Division
Assistant Commissioner for Patents
Mail Stop PCT
P.O. Box 1450
Alexandria, VA 22313-1450

Re: International Application No. PCT/US03/02353
Applicants: CORIXA CORPORATION *et al.*
Inventors: Gaiger *et al.*
Filed: 22 January 2003
Express Mail Label No.: EV 332 017 306 US
Date of Mailing: 14 August 2003
Our File No.: 14058-14402P

Dear Officer:

Enclosed are the Chapter II Demand with ten (10) substitute pages 15, 20, 20a, 22, 161, 167, 167a, 169, 171, and 234 of the Specification, and fifteen (15) sheets of Formal Drawings (Figs. 1-8), submitted as Amendments under Article 34. The only changes were corrections to typographical errors and the insertions of SEQ ID: NOs. that do not include matter which go beyond the disclosure in the international application as filed.

It is hereby stated that "the information recorded on the computer readable form is identical to the written sequence listing" and does not include matter which goes beyond the disclosure in the international application as filed.

Thank you for your attention to this matter.

Respectfully submitted,


Carol A. Fang

CAF:kji

Enclosures: Chapter II Demand
Substitute pages 15, 20, 20a, 22, 161, 167, 167a, 169, 171, and 234
Fifteen (15) sheets of Formal Drawings (Figs. 1-8)
One hundred twenty (120) pages of Sequence Listing
Diskette and Statement
Transmittal Letter
Postcard

The demand must be filed directly with the competent International Preliminary Examining Authority or, if two or more Authorities are competent, with the one chosen by the applicant. The full name or two-letter code of that Authority may be indicated by the applicant on the line below:

IPEA/ US

PCT

CHAPTER II

DEMAND

under Article 31 of the Patent Cooperation Treaty:

The undersigned requests that the international application specified below be the subject of international preliminary examination according to the Patent Cooperation Treaty and hereby elects all eligible States (except where otherwise indicated).

For International Preliminary Examining Authority use only

Identification of IPEA		Date of receipt of DEMAND	
Box No. I IDENTIFICATION OF THE INTERNATIONAL APPLICATION		Applicant's or agent's file reference 14058-14402P	
International application No. PCT/US03/02353	International filing date (day/month/year) 22 January 2003 (22.01.03)	(Earliest) Priority date (day/month/year) 22 January 2002 (22.01.02)	
Title of invention COMPOSITIONS AND METHODS FOR THE DETECTION, DIAGNOSIS AND THERAPY OF HEMATOLOGICAL MALIGNANCIES			
Box No. II APPLICANT(S)			
Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.) CORIXA CORPORATION 1124 Columbia Street, Suite 200 Seattle, Washington 98104 United States of America		Telephone No.: 206.754.5711	
		Facsimile No.: 206.754.5994	
		Teleprinter No.:	
		Applicant's registration No. with the Office	
State (that is, country) of nationality: US		State (that is, country) of residence: US	
Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.) GAIGER, Alexander Doeblinger Hauptstrasse 62/14 A-1190 Vienna Austria			
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Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.) ALGATE, Paul, A. 580 Kalmia Place, NW Issaquah, Washington 98027 United States of America			
State (that is, country) of nationality: GB		State (that is, country) of residence: US	
<input checked="" type="checkbox"/> Further applicants are indicated on a continuation sheet.			

Continuation of Box No. II APPLICANT(S)

If none of the following sub-boxes is used, this sheet should not be included in the demand.

Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)

MANNION, Jane
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State (that is, country) of nationality:

US

State (that is, country) of residence:

US



Further applicants are indicated on a continuation sheet.

Continuation of Box No. II APPLICANT(S)

If none of the following sub-boxes is used, this sheet should not be included in the demand.

Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)

CARTER, Lauren
7143 Beach Drive, SW
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State (that is, country) of residence:

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State (that is, country) of nationality:

State (that is, country) of residence:

Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)

State (that is, country) of nationality:

State (that is, country) of residence:

☐

Further applicants are indicated on a continuation sheet.

Box No. III AGENT OR COMMON REPRESENTATIVE; OR ADDRESS FOR CORRESPONDENCEThe following person is ☒ agent ☐ common representativeand ☒ has been appointed earlier and represents the applicant(s) also for international preliminary examination.☐ is hereby appointed and any earlier appointment of (an) agent(s)/common representative is hereby revoked.☐ is hereby appointed, specifically for the procedure before the International Preliminary Examining Authority, in addition to the agent(s)/common representative appointed earlier.Name and address: *(Family name followed by given name; for a legal entity, full official designation.
The address must include postal code and name of country.)*

Carol A. Fang
 TOWNSEND AND TOWNSEND AND CREW LLP
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 United States of America

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415-576-0200

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Teleprinter No.:

Agent's registration No. with the Office

48,631

☐ Address for correspondence: Mark this check-box where no agent or common representative is/has been appointed and the space above is used instead to indicate a special address to which correspondence should be sent.**Box No. IV BASIS FOR INTERNATIONAL PRELIMINARY EXAMINATION****Statement concerning amendments:***

1. The applicant wishes the international preliminary examination to start on the basis of:

☒ the international application as originally filedthe description ☐ as originally filed☒ as amended under Article 34the claims ☒ as originally filed☐ as amended under Article 19 (together with any accompanying statement)☐ as amended under Article 34the drawings ☐ as originally filed☒ as amended under Article 342. ☐ The applicant wishes any amendment to the claims under Article 19 to be considered as reversed.3. ☐ The applicant wishes the start of the international preliminary examination to be postponed until the expiration of 20 months from the priority date unless the International Preliminary Examining Authority receives a copy of any amendments made under Article 19 or a notice from the applicant that he does not wish to make such amendments (Rule 69.1(d)). *(This check-box may be marked only where the time limit under Article 19 has not yet expired.)*

* Where no check-box is marked, international preliminary examination will start on the basis of the international application as originally filed or, where a copy of amendments to the claims under Article 19 and/or amendments of the international application under Article 34 are received by the International Preliminary Examining Authority before it has begun to draw up a written opinion or the international preliminary examination report, as so amended.

Language for the purposes of international preliminary examination: ENGLISH☒ which is the language in which the international application was filed.☐ which is the language of a translation furnished for the purposes of international search.☐ which is the language of publication of the international application.☐ which is the language of the translation (to be) furnished for the purposes of international preliminary examination.**Box No. V ELECTION OF STATES**The applicant hereby elects all eligible States *(that is, all States which have been designated and which are bound by Chapter II of the PCT)*

excluding the following States which the applicant wishes not to elect:

Box No. VI CHECK LIST

The demand is accompanied by the following elements, in the language referred to in Box No. IV, for the purposes of international preliminary examination:

- | | | | |
|--|---|----|--------|
| 1. translation of international application | : | | sheets |
| 2. amendments under Article 34 | : | 25 | sheets |
| 3. copy (or, where required, translation) of amendments under Article 19 | : | | sheets |
| 4. copy (or, where required, translation) of statement under Article 19 | : | | sheets |
| 5. letter | : | 1 | sheet |
| 6. other (<i>specify</i>) | : | | sheets |

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received not received

<input type="checkbox"/>	<input type="checkbox"/>
<input type="checkbox"/>	<input type="checkbox"/>
<input type="checkbox"/>	<input type="checkbox"/>
<input type="checkbox"/>	<input type="checkbox"/>
<input type="checkbox"/>	<input type="checkbox"/>
<input type="checkbox"/>	<input type="checkbox"/>

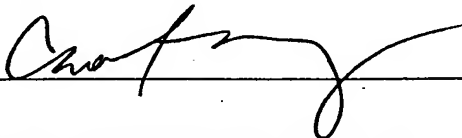
The demand is also accompanied by the item (s) marked below:

- | | |
|--|---|
| 1. <input checked="" type="checkbox"/> fee calculation sheet | 5. <input type="checkbox"/> statement explaining lack of signature |
| 2. <input type="checkbox"/> original separate signed power of attorney | 6. <input checked="" type="checkbox"/> sequence listing in computer readable form |
| 3. <input type="checkbox"/> original general power of attorney; | 7. <input type="checkbox"/> tables in computer readable form related to sequence listings |
| 4. <input type="checkbox"/> copy of general power of attorney; reference number, if any: | 8. <input checked="" type="checkbox"/> other (<i>specify</i>) Transmittal Letter, Article 34 Amendment with ten (10) substitute pages 15, 20, 20a, 22, 161, 167, 167a, 169, 171, and 234; fifteen (15) sheets of Formal Drawings (Figs. 1-8); Statement; Sequence Listing (120 pages), and Diskette; and Postcard |

Box No. VII SIGNATURE OF APPLICANT, AGENT OR COMMON REPRESENTATIVE

Next to each signature, indicate the name of the person signing and the capacity in which the person signs (if such capacity is not obvious from reading the demand).

X



Carol A. Fang
TOWNSEND AND TOWNSEND AND CREW LLP
USPTO Reg. No. 48,631
Applicants' Agent

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- | | |
|--|---|
| 1. Date of actual receipt of DEMAND: | |
| 2. Adjusted date of receipt of demand due to CORRECTIONS under Rule 60.1(b): | |
| 3. <input type="checkbox"/> The date of receipt of the demand is AFTER the expiration of 19 months from the priority date and item 4 or 5, below, does not apply. | <input type="checkbox"/> The applicant has been informed accordingly. |
| 4. <input type="checkbox"/> The date of receipt of the demand is WITHIN the period of 19 months from the priority date as extended by virtue of Rule 80.5. | |
| 5. <input type="checkbox"/> Although the date of receipt of the demand is after the expiration of 19 months from the priority date, the delay in arrival is EXCUSED pursuant to Rule 82. | |

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Demand received from IPEA on:

PCT

FEE CALCULATION SHEET

Annex to the Demand

For International Preliminary Examining Authority use only

International application No. PCT/US03/02353	
Applicant's or agent's file reference 14058-14402P	Date stamp of the IPEA
Applicant CORIXA CORPORATION et al.	
CALCULATION OF PRESCRIBED FEES	
1. Preliminary examination fee	<div style="border: 1px solid black; padding: 2px; display: inline-block;">\$490.00</div> <div style="border: 1px solid black; padding: 2px; display: inline-block; margin-left: 10px;">P</div>
2. Handling fee (<i>Applicants from certain States are entitled to a reduction of 75% of the handling fee. Where the applicant is (or all applicants are) so entitled, the amount to be entered at H is 25% of the handling fee.</i>)	<div style="border: 1px solid black; padding: 2px; display: inline-block;">\$172.00</div> <div style="border: 1px solid black; padding: 2px; display: inline-block; margin-left: 10px;">H</div>
3. Total of prescribed fees Add the amounts entered at P and H and enter total in the TOTAL box	<div style="border: 1px solid black; padding: 2px; display: inline-block;">\$662.00</div>
<div style="border: 1px solid black; padding: 2px; display: inline-block;">TOTAL</div>	
MODE OF PAYMENT	
<div style="display: flex; justify-content: space-between;"> <div style="width: 45%;"> <input checked="" type="checkbox"/> authorization to charge deposit account with the IPEA (see below) </div> <div style="width: 45%;"> <input type="checkbox"/> cash </div> </div>	
<div style="display: flex; justify-content: space-between;"> <div style="width: 45%;"> <input type="checkbox"/> cheque </div> <div style="width: 45%;"> <input type="checkbox"/> revenue stamps </div> </div>	
<div style="display: flex; justify-content: space-between;"> <div style="width: 45%;"> <input type="checkbox"/> postal money order </div> <div style="width: 45%;"> <input type="checkbox"/> coupons </div> </div>	
<div style="display: flex; justify-content: space-between;"> <div style="width: 45%;"> <input type="checkbox"/> bank draft </div> <div style="width: 45%;"> <input type="checkbox"/> other (specify): </div> </div>	
AUTHORIZATION TO CHARGE (OR CREDIT) DEPOSIT ACCOUNT <i>(This mode of payment may not be available at all IPEAs)</i>	
The IPEA/ <u>US</u>	
<input checked="" type="checkbox"/> is hereby authorized to charge the total fees indicated above to my deposit account.	
<input checked="" type="checkbox"/> (this check-box may be marked only if the conditions for deposit accounts of the IPEA so permit) is hereby authorized to charge any deficiency or credit any overpayment in the total fees indicated above to my deposit account.	
20-1430 Deposit Account Number	14 August 2003 Date (day/month/year)
<div style="display: flex; align-items: center;"> <div style="margin-right: 10px;"> </div> <div> Signature Carol A. Fang </div> </div>	

See Notes to the fee calculation sheet

56, 58, 61, 63, 66, 71, 74, 76, 82, 84, 87, 101, 104, 107, 109, 114, 117, or 119-121 herein.

[38] In still other embodiments, the preferred peptides and polypeptides of the present invention comprise a sequence of at least about 100, 125, 150, 175, 200, 225, 250, 275, 300, 325, 350, 375, or 400 or more contiguous amino acids as disclosed in any one or more of the peptides encoded by any one of SEQ ID NOS: 1-3, 5, 7, 9, 11, 13-14, 16-17, 19-20, 22-25, 27-28, 30-31, 33-34, 36, 38-39, 41-42, 44, 46-47, 49, 51, 53, 55, 57, 59-60, 62, 64-65, 67-70, 72-73, 75, 77-81, 83, 85-86, 88-100, 102-103, 105-106, 108, 110-113, 115-116, 118, or 124 or disclosed in any one of SEQ ID NOS: 4, 6, 8, 10, 12, 15, 18, 21, 26, 29, 32, 35, 37, 40, 43, 45, 48, 50, 52, 54, 56, 58, 61, 63, 66, 71, 74, 76, 82, 84, 87, 101, 104, 107, 109, 114, 117, or 119-121 herein.

[39] The polypeptides of the invention typically will comprise at least a first contiguous amino acid sequence according to any one of the peptides encoded by any one of the above polynucleotides or disclosed in any one of SEQ ID NOS: 10,471-10,474; SEQ ID NO: 10,481; SEQ ID NOS: 10,599 – 10,819; SEQ ID NOS: 10,820-10,842; SEQ ID NOS: 10,849-10,908; and SEQ ID NOS: 10,909-10,968 of co-pending application USSN 10/057,475, but may also, optionally comprise at least a second, at least a third, or even at least a fourth or greater contiguous amino acid sequence according to any one of the peptides encoded by any one of SEQ ID NOS: 1-3, 5, 7, 9, 11, 13-14, 16-17, 19-20, 22-25, 27-28, 30-31, 33-34, 36, 38-39, 41-42, 44, 46-47, 49, 51, 53, 55, 57, 59-60, 62, 64-65, 67-70, 72-73, 75, 77-81, 83, 85-86, 88-100, 102-103, 105-106, 108, 110-113, 115-116, 118, or 124 or disclosed in any one of SEQ ID NOS: 4, 6, 8, 10, 12, 15, 18, 21, 26, 29, 32, 35, 37, 40, 43, 45, 48, 50, 52, 54, 56, 58, 61, 63, 66, 71, 74, 76, 82, 84, 87, 101, 104, 107, 109, 114, 117, or 119-121. A single polypeptide may contain only a single coding region, or alternatively, a single polypeptide may comprise a plurality of identical or distinctly different contiguous amino acid sequences in accordance with any one of the peptides encoded by any one of SEQ ID NOS: 1-3, 5, 7, 9, 11, 13-14, 16-17, 19-20, 22-25, 27-28, 30-31, 33-34, 36, 38-39, 41-42, 44, 46-47, 49, 51, 53, 55, 57, 59-60, 62, 64-65, 67-70, 72-73, 75, 77-81, 83, 85-86, 88-100, 102-103, 105-106, 108, 110-113, 115-116, 118, or 124 or disclosed in any one of SEQ ID NOS: 4, 6, 8, 10, 12, 15, 18, 21, 26, 29, 32, 35, 37, 40, 43, 45, 48, 50, 52, 54, 56, 58, 61, 63, 66, 71, 74, 76, 82, 84, 87, 101, 104, 107, 109, 114, 117, or 119-121. In fact, the polypeptide may comprise a plurality of the same contiguous amino acid sequences, or they may comprise one or more different contiguous amino acid sequences of any of the peptides encoded by any one

- [50] FIG. 3 illustrates a schematic outline of the general protocol for in vitro whole gene CD4⁺ T cell priming procedure used to generate antigen-specific lines and to identify clones of interest.
- [51] FIG. 4 illustrates the panel of probes used to identify cDNAs that are overexpressed in lymphoma cells.
- [52] FIG. 5 lists the antigens that have similar tissue expression profiles as the known therapeutics, CD20 and CD52.
- [53] FIG. 6 illustrates the results of the TMPred report for Ly1484 long (SEQ ID NO:120) and Ly1484 short (SEQ ID NO:121).
- [54] FIG. 7 illustrates the results of the TSITES analysis of Ly1484 long (SEQ ID NO:120).
- [55] FIG. 8 illustrates the results of the TSITES analysis of Ly1484 short (SEQ ID NO:121).
- [56] SEQ ID NO:1 is a full-length cDNA for Ly1728P.
- [57] SEQ ID NO:2 is a full-length protein sequence for Ly1728P.
- [58] SEQ ID NO:3 is a full-length cDNA sequence of Ly1732P.
- [59] SEQ ID NO:4 is a full-length protein of Ly1732P.
- [60] SEQ ID NO:5 is a full length cDNA sequence of Ly1888P.
- [61] SEQ ID NO:6 is a full length protein sequence of Ly1888P.
- [62] SEQ ID NO:7 is a full length cDNA sequence of Ly1452_His-tag-fusion.
- [63] SEQ ID NO:8 is a full length protein sequence of Ly1452_His-tag-fusion.
- [64] SEQ ID NO:9 is a full length cDNA sequence of Ly1452P, splice variant 1.
- [65] SEQ ID NO:10 is a full length protein sequence of Ly1452P, splice variant 1.
- [66] SEQ ID NO:11 is a full length cDNA sequence of Ly1452P, splice variant 2.
- [67] SEQ ID NO:12 is a full length protein sequence of Ly1452P, splice variant 2.
- [68] SEQ ID NO:13 is a partial cDNA sequence of Ly1462P.
- [69] SEQ ID NO:14 is a full length cDNA sequence of Ly1462P.
- [70] SEQ ID NO:15 is a full length protein sequence of Ly1462P.
- [71] SEQ ID NO:16 is a partial cDNA sequence of Ly1484P.
- [72] SEQ ID NO:17 is a full length cDNA sequence of Ly1484P.
- [73] SEQ ID NO:18 is a full length protein sequence of Ly1484P.
- [74] SEQ ID NO:19 is a partial cDNA sequence of Ly1486P.
- [75] SEQ ID NO:20 is a full length cDNA sequence of Ly1486P.
- [76] SEQ ID NO:21 is a full length protein sequence of Ly1486P.

[77] SEQ ID NO:22 is a partial cDNA sequence of Ly1677P.

[78] SEQ ID NO:23 is a partial cDNA sequence of Ly1682P.

- [113] SEQ ID NO:58 is a full-length protein sequence of CD138.
- [114] SEQ ID NO:59 is a partial cDNA sequence of CD22.
- [115] SEQ ID NO:60 is a full-length cDNA sequence of CD22.
- [116] SEQ ID NO:61 is a full-length protein sequence of CD22.
- 5 [117] SEQ ID NO:62 is a partial cDNA sequence of CD79beta.
- [118] SEQ ID NO:63 is a partial protein sequence of CD79beta.
- [119] SEQ ID NO:64 is a partial cDNA sequence of CD79beta.
- [120] SEQ ID NO:65 is a full-length cDNA sequence of CD79beta.
- [121] SEQ ID NO:66 is a full-length protein sequence of CD79beta.
- 10 [122] SEQ ID NO:67 is a partial cDNA sequence of Ly1450P.
- [123] SEQ ID NO:68 is a partial cDNA sequence of Ly1450P.
- [124] SEQ ID NO:69 is a partial cDNA sequence of Ly1451P.
- [125] SEQ ID NO:70 is a partial cDNA sequence of Ly1451P.
- [126] SEQ ID NO:71 is a partial protein sequence of Ly1451P.
- 15 [127] SEQ ID NO:72 is a partial cDNA sequence of Ly1454P.
- [128] SEQ ID NO:73 is a full-length cDNA sequence of Ly1454P.
- [129] SEQ ID NO:74 is a full-length protein sequence of Ly1454P.
- [130] SEQ ID NO:75 is a partial cDNA sequence of Ly1485P.
- [131] SEQ ID NO:76 is a partial protein sequence of Ly1485P.
- 20 [132] SEQ ID NO:77 is a partial cDNA sequence of Ly1485P.
- [133] SEQ ID NO:78 is a partial cDNA sequence of Ly1500P.
- [134] SEQ ID NO:79 is a full-length cDNA sequence of Ly1500P, splice variant 1.
- [135] SEQ ID NO:80 is a full-length protein sequence of Ly1500P, splice variant 1.
- [136] SEQ ID NO:81 is a full-length cDNA sequence of Ly1500P, splice variant 2.
- 25 [137] SEQ ID NO:82 is a full-length protein sequence of Ly1500P, splice variant 2.
- [138] SEQ ID NO:83 is a full-length cDNA sequence of Ly1500P, splice variant 3.
- [139] SEQ ID NO:84 is a full-length protein sequence of Ly1500P, splice variant 3.
- [140] SEQ ID NO:85 is a partial cDNA sequence of Ly1516P.
- [141] SEQ ID NO:86 is a full-length cDNA sequence of Ly1516P, splice variant 1.
- 30 [142] SEQ ID NO:87 is a full-length protein sequence of Ly1516P, splice variant 1.
- [143] SEQ ID NO:88 is a partial cDNA sequence of Ly1516P, splice variant 2.
- [144] SEQ ID NO:89 is a partial cDNA sequence of Ly1516P, splice variant 3.
- [145] SEQ ID NO:90 is a partial cDNA sequence of Ly1678P.
- [146] SEQ ID NO:91 is a partial cDNA sequence of Ly1678P.

mRNA, and the reproducibility of the technology may be ensured by including duplicated control cDNA elements at different locations.

[620] Analysis of hematological malignancy subtracted clones by microarray analyses on a variety of microarray chips identified the sequences set forth in SEQ ID NO:1 through SEQ ID NO:664 of co-pending application USSN 09/796,692 as being at least two-fold overexpressed in hematological malignancies versus normal tissues.

5.3 EXAMPLE 3 – POLYNUCLEOTIDE AND POLYPEPTIDE COMPOSITIONS: BRIEF DESCRIPTION OF THE cDNA CLONES AND OPEN READING FRAMES IDENTIFIED BY SUBSTRUCTIVE HYBRIDIZATION AND MICROARRAY ANALYSIS

[621] Table 7 in co-pending application USSN 09/796,692 lists the sequences of the polynucleotides obtained during the analyses of the present invention. Shown are the 664 polynucleotide sequences, along with their clone name identifiers, as well as the serial number and filing date of the priority provisional patent application in which the clone was first identified. Also listed in Table 7 are the TCL-1 DNA and protein (SEQ ID NOS:665 and 666) and coronin 1A DNA and protein (SEQ ID NOS:667 and 668).

[622] Table 8 in co-pending application USSN 09/796,692 identifies the putative open reading frames obtained from analyses of the cDNA sequences obtained in SEQ ID NO:1-SEQ ID NO:664 in the co-pending application. Shown are the sequence identifiers, the clone name and translation frame, and the start and stop nucleotides in the corresponding DNA sequence used to generate the polypeptide sequence of the open reading frame (SEQ ID NOS:669-2532).

[623] Table 9 in co-pending application USSN 09/796,692 identifies an additional set of particular hematological malignancy-related cDNA sequences that were obtained using the subtractive library and microarray methods as described above. These sequences, designated SEQ ID NO:2533-SEQ ID NO:9597 in the co-pending application USSN 09/796,692, are shown in the Table along with the original clone name, and the serial number and filing date of the priority provisional application in which the clone was first described.

5.4 EXAMPLE 4 – ADDITIONAL ANALYSIS OF cDNA CLONES AND ORFS IDENTIFIED BY SUBSTRUCTIVE HYBRIDIZATION AND MICROARRAY ANALYSIS

[624] This example describes microarray analysis of leukemia tumor- and tissue-specific cDNAs.

[660] For Ly1859P, amino acid residues 128-144, 293-311, 408-425, 435-454, 465-483, 516-533, 290-311; 435-456, and 507-528 of SEQ ID NO:107 were identified as putative transmembrane domains.

[661] For Ly1866P, amino acids 47-65 and 50-71 of SEQ ID NO:109 were identified as putative transmembrane domains.

[662] For Ly669S, amino acids 489-505, 13-29, 38-57, 73-89, 94-114, 252-268, 307-324, 329-346, 489-509, 4-25, and 486-507 of SEQ ID NO:114 were identified as putative transmembrane domains.

[663] For Ly672S, amino acids 11-27, 284-300, 325-341, 345-361, 407-423, 7-28, 102-118, 174-198, 283-299, 325-341, 347-383, 403-423, 431-454, 473-492, 11-32, 286-307, 322-343, 345-366, 404-425, 430-451, and 469-490 of SEQ ID NO:117 were identified as putative transmembrane domains.

[664] For Ly675S, amino acids 154-170, 187-203, 428-444, 518-534, 846-862, 81-97, 155-172, 235-251, 374-391, 428-444, 477-195, 520-542, 539-573, 694-714, 807-823, 843-862, 50-71, 77-98, 145-166, 518-539, 802-823, and 845-866 of SEQ ID NO:119 were identified as putative transmembrane domains.

5.8 EXAMPLE 8 – REALTIME PCR ANALYSIS TO IDENTIFY ANTIGENS OVEREXPRESSED IN CHRONIC LYMPHOCYTIC LEUKEMIA AND MULTIPLE MYELOMA

[665] Overexpression of candidate antigens in chronic lymphocytic leukemia (CLL) and multiple myeloma (MM) was confirmed by RealTime PCR.

[666] Real-time PCR evaluates the level of PCR product accumulation during amplification (*see, e.g.,* Gibson *et al.*, Genome Research 6:995-1001 (1996); Heid *et al.*, Genome Research 6:986-994 (1996)). RealTime PCR permits quantitative evaluation of mRNA levels in multiple samples. Briefly, mRNA is extracted from tumor and normal tissue and cDNA is prepared using standard techniques. Real-time PCR is performed, for example, using a Perkin Elmer/Applied Biosystems (Foster City, CA) 9700 Prism instrument. Matching primers are designed for genes of interest using, for example, the primer express program provided by Perkin Elmer/Applied Biosystems (Foster City, CA). Optimal concentrations of primers and probes are initially determined by those of ordinary skill in the art, and control (*e.g.,* β -actin) primers and probes are obtained commercially from, for example, Perkin

Elmer/Applied Biosystems (Foster City, CA). To quantitate the amount of specific RNA in a sample, a standard curve is generated using a plasmid containing the gene of

Ly1866P	yes	yes
Ly1867P	yes	no
Ly1868P	yes	no
Ly1886P	yes	no
Ly669S	yes	yes
Ly672S	yes	yes
Ly675S	yes	yes

[667] These sequences can conveniently be used to diagnose, treat, and prevent malignant diseases that overexpress these genes, including multiple myeloma, B-cell lymphomas, and B-CLL. For example, monoclonal antibodies, including humanized monoclonal antibodies can be used for diagnosis and therapy of disorders associated with expression of antigens overexpressed in hematological malignancies.

5.9 EXAMPLE 9 - SEQUENCE ANALYSES, EXPRESSION ANALYSES, AND STRUCTURE ANALYSES OF OTHER ANTIGENS WITH SIMILAR EXPRESSION PROFILES AS CD20 & CD52

Summary of Results

Antigen	Sequence Analysis
Ly1728P	FOAP-12 ("novel gene over-expressed in macrophages")
Ly1732P	B-cell maturation factor (BCM), tumor necrosis factor receptor superfamily, member 17. BCM bins to TALL-1, a member of the TNF family.
Ly1888P	anti-Fas-induced apoptosis protein (TOSO); experimentally shown to be expressed on the cell surface.
Ly 1452P	anti-Fas-induced apoptosis protein (TOSO); experimentally shown to be expressed on the cell surface.
Ly1462P	Human Epstein-Barr virus complement receptor type II
Ly1484P	KIAA1607 (cDNA sequence present in GenBank).
Ly1486P	Fc fragment of IgE, low affinity II receptor.
Ly1677P	novel
Ly1682P	novel
Ly1693P	Chemokine receptor CXCR4
Ly1697P	novel
Ly1715P	lectin-like NK cell receptor
Ly1727P	Splice variants of the hpim-2 gene (homologs of the mouse pim-2 oncogene). Predicted to be a serine threonine kinase with a role in cell proliferation.
Ly1905P	Splice variants of the hpim-2 gene (homologs of the mouse pim-2 oncogene). Predicted to be a serine threonine kinase with a role in cell proliferation.
Ly1885P	An apparent splice form of the cell cycle progression 8 protein: one of a family of proteins involved in restoration of cell cycle progression (by blocking arrest in G1 phase).
Ly663S	leukocyte surface antigen CD37

(template #1076101.8; SEQ ID NO:124 that contained all 240bp of Ly1451. This template consisted of sequences from 6 clones, of which 2 (33%) were derived from hematologic/immune tissue libraries. Template #1076101.8 was part of a bin containing 11 templates derived from a total of 104 clones, of which 12 (9%) were derived from

[669] This sequence (SEQ ID NO:124) was used to search further public databases but no additional sequences were obtained. However, these searches indicate this sequence is a human endogenous retroviral sequence (HERV) encoding polypeptides corresponding to portions of the integrase and envelope genes. A single ORF with an ATG translational start site is contained in the forward read of LS1076101.8

[670] The polypeptide encoded by this ORF (SEQ ID NO:124) is not predicted to have a transmembrane domain.

5.11 EXAMPLE 11 —EXPRESSION OF LY1452 LYMPHOMA ANTIGENS ENCODED BY A SPECIFIC GENE, LY1452, ASSOCIATED WITH B CELL LEUKEMIAS, LYMPHOMAS AND MULTIPLE MYELOMAS

[671] Recombinantly expressed Ly1452 antigens were constructed to allow for quick and easy purification of the protein.

[672] The open reading frame for Ly1452 was PCR amplified and subcloned into a modified pET28 vector with a His tag in-frame and recombinantly expressed in *E. coli* (His-Ly1452: SEQ ID NO:7 (nt), SEQ ID NO:8 (protein)).

Ly1452P expression in *E. coli*

[673] The open reading frame of the LS coding region was PCR amplified with the following primers:

PDM-797 5' gtgtcacaatctacagtcaggcaggattctcc 3' T_m 64°C
(SEQ ID NO:122)
PDM-799 5' gttatgtagcggccgcttatcatgttgctgcagag 3' T_m 67°C
(SEQ ID NO:123)

[674] Using the following conditions:

10µl 10X Herculanase buffer
1 µl 10mM dNTPs
2µl 10µM each oligo
83µl sterile water
1.0µl Herculanase DNA polymerase (Stratagene, La Jolla, CA)
50 ng DNA

atctccccagagctgtctggggccatgggtgggtgggggacgtgctctgtctggccactgttctgaccagcc
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119>Ly675S, KIAA0906 protein, partial protein

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PPSGLWSPAYASH

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<211> 1270

<212> PRT

<213> Homo sapiens

<400> 120

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Met	Thr	Ser	Gly	Gly	Asp	Ala	Ala	Met	Phe	Arg	Asp	Gly	Lys	Glu	Pro
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Gln	Pro	Ser	Ala	Glu	Ala	Ala	Ala	Ala	Pro	Ser	Leu	Ala	Asn	Ile	Ser
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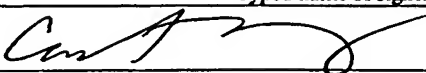
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application no.		filed on							

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